

# Cloning, nucleotide sequence and transfer of genes for the B800-850 light harvesting complex of *Rhodobacter sphaeroides*

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The genes that encode the  $\alpha$  and  $\beta$  polypeptides of the major light-harvesting complex of *Rhodobacter sphaeroides*, B800-850, have been cloned and sequenced through the use of oligonucleotides based upon the known polypeptide sequences. These genes, *pucA* and *B*, are transcribed in the order *B*, *A*, are of 150 and 164 nucleotides respectively, and are separated by a spacer region of 14 nucleotides. Transfer of these genes to mutant M21 lacking the B800-850 complex has been accomplished and absorbance spectra of recombinant strains M2181 and M2184 show that expression of *pucA* and *B* is comparable to levels found in the wild type.

Photosynthesis; Light harvesting; Gene cloning; Gene transfer; Photosynthetic bacteria

## 1. INTRODUCTION

The light-harvesting antenna of the photosynthetic bacterium *Rhodobacter sphaeroides* consists of the major complex, B800-850, arranged around a cluster formed by B875 antenna and approximately four reaction centres [1]. Within the photosynthetic membrane, approx. 1000 bacteriochlorophyll molecules are connected to form a domain for efficient energy transfer [2,3] and excitation energy is eventually trapped by a reaction centre where the primary photochemical event occurs.

The bacteriochlorophyll and carotenoid pigment

of B800-850 and B875 are non-covalently bound to small polypeptides designated  $\alpha$  and  $\beta$ . These simple antennae have been extensively characterised [4–6] and they are therefore attractive model systems for further studies involving manipulation of antenna structure through mutagenesis of *pucA* and *B* genes.

Here, the genes encoding the B800-850  $\alpha$  and  $\beta$  polypeptides, *pucA* and *B*, have been cloned and sequenced. These genes have been transferred to mutant M21 which lacks the B800-850 antenna, resulting in the restoration of a wild-type light-harvesting system.

## 2. MATERIALS AND METHODS

### 2.1. Oligonucleotide probe

A mixed 23-base oligonucleotide deduced from the published amino-terminal sequence of the  $\alpha$ -subunit of the B800-850 complex [7] was made on an Applied Biosystems 380B synthesiser using methyl phosphoramidite. A 17-base mixed oligonucleotide was also made, but corresponding

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A preliminary report of this work was presented at the VII International Congress on Photosynthesis, Rhode Island, USA, August, 1986. Similar results were presented by P.J. Kiley, T.J. Donohue and S. Kaplan

to the opposite DNA strand. The oligonucleotides were radiolabelled using polynucleotide kinase; probing was as described by Woods [8].

## 2.2. Molecular cloning

A genomic library of *R. sphaeroides* DNA (average size 5 kb) was prepared in shuttle vector pNH2 (C.N.H., unpublished) in *E. coli* DH5. Plasmid DNA was prepared from positively hybridising clones using a Triton lysis method [10]. Following restriction analysis and Southern transfer [11] the nitrocellulose filters were probed with labelled oligonucleotides. DNA cloned was cloned into M13 mp18 and sequenced [12] using the universal 17-base primer and the mixed 17-mer (above).

## 2.3. Mutagenesis

Mutant M21, which lacks the B800-850 antenna complex, was isolated as a result of chemical mutagenesis of wild-type strain NC1B 8253 by *N*-nitrosoguanidine.

## 2.4. Plasmid transfer

Plasmids pMA81 and pMA84 were transferred from *E. coli* to mutant M21 using a mobilisation system based upon plasmid RP4 (C.N.H. and Olsen, J.D., in preparation). Amongst the pale background, some darker and larger colonies were obtained after 10 days of anaerobic photosynthetic growth and absorbance spectra of cultures derived from these colonies were used to investigate whether mutant M21 had acquired the B800-850 complex.

# 3. RESULTS AND DISCUSSION

## 3.1. Cloning and nucleotide sequencing of *pucA* and *B* genes

The sequence of the mixed oligonucleotide probe is shown in fig.1; it has a degeneracy of 2028. 6000 colonies of the gene library were screened and four positively hybridising clones were found which were used to prepare plasmid DNA (pMA81-4). pMA82, 83 and 84 had identical restriction patterns with *EcoRI*, *HindIII* and *PstI* and had inserts of approx. 7 kb, whereas pMA81 had an insert of 3.75 kb (fig.2). Southern hybridisation revealed that a 1.1 kb *BamHI* fragment of pMA81 must contain at least part of the

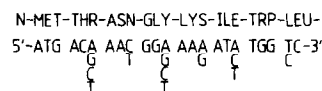


Fig.1. 23-base oligonucleotide deduced from the N-terminal sequence of the B800-850  $\alpha$  polypeptide.

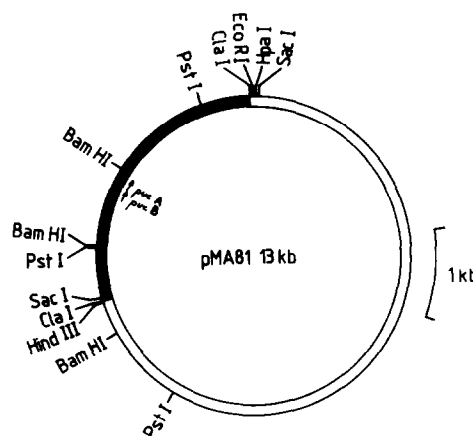


Fig.2. pMA81 showing 1.1 kb *BamHI* fragment containing *pucA* and *B* genes.

*pucA* gene. After recloning into M13 mp18 and sequencing it was apparent that in one of the possible orientations, all of the *pucA* gene had been identified by comparison of the deduced polypeptide sequence with the amino acid sequence determined by Theiler et al. [7]. Through the use of a second oligonucleotide primer complementary to part of the *pucB* gene, the sequencing of both genes was completed and is shown in fig.3. Although the sequence was translated into six possible reading frames, one is presented which is in complete agreement with that published by Theiler et al. [7], with the exception of an exchange of isoleucine for leucine at position 37 in the  $\beta$ -chain in our sequence. This difference is not expected to have any effects on the conformation or function of the polypeptide (Theiler, R., personal communication), and we attribute it to a difference between our wild-type strain (NCIB 8253) and that used by Theiler (2.4.1). As has already been found for *R. capsulatus* [13], the genes are transcribed in the order *pucB*, *A* and each gene is preceded by sequences which are possible sites for ribosome binding [14]. These are indicated in fig.3. There is a space region of 14 nucleotides between the genes.

**β** → MET THR ASP ASP LEU ASN

GCCTAGCCACACCCCTCGATTTCACATTGGAGACGCACATG ACT GAC GAT CTC AAC  
 CCGGATCGCTGTGGCAGCTAAATGATACCTCTGCGTGTAC TGA CTG CTA GAG TTG

LYS VAL TRP PRO SER GLY LEU THR VAL ALA GLU ALA GLU GLU VAL HIS  
 AAA GTC TGG CCG AGC GGC CTC ACC GTT GCC GAA GCC GAA GAA GTT CAT  
 TTT CAG ACC GGC TCG CCG GAG TGG CAA CCG CTT CCG CTT CAA GAT

LYS GLN LEU ILE LEU GLY THR ARG VAL PHE GLY GLY MET ALA LEU LEU  
 AAG CAA CTC ATC CTC GGC ACC GCG GTC TTC GGT GGC ATG GCT CTG CTC  
 TTC GTT GAG TAG GAG CCG TGG GCG CAG AAG CCA CCG TAC CBA GAC GAG

ALA HIS PHE LEU ALA ALA ALA ALA THR PRO TRP LEU GLY \*\*\*  
 GCG CAC TTC CTC GCC GGC GCT GCG ACC CCC TGG CTC GGC TGA TATGA  
 CGC GTG AAG GAG CCG CCG CCG TGG GGG ACC GAG CCG ACT ATACT

**α** → MET THR ASN GLY LYS ILE TRP LEU VAL VAL LYS PRO THR

GAGACTGACATG ACC AAC GGC AAA ATC TGG CTC GTG GTG AAA CCG ACC  
 CTCTGACTGTAC TGG TTG CCG TTT TAG ACC GAG CAC CAC TTT GGC TGG

VAL GLY VAL PRO LEU PHE LEU SER ALA ALA VAL ILE ALA SER VAL VAL  
 GTC GGC GTT CCG CTG TTC CTC AGC GCT GCC GTC ATC GCC TCC GTC GTT  
 CAG CCG CAA GGC GAC AAG GAG TCG CGA CCG CAG TAG CCG AGG CAG CAA

ILE HIS ALA ALA VAL LEU THR THR THR THR TRP LEU PRO ALA TYR TYR  
 ATC CAC GCT GCT GTG CTG ACG ACC ACC TGG CTG CCG GGC TAC TAC  
 TAG GTG CGA CGA CAC GAC TGC TGG TGG TGG ACC GAC GGG CCG ATG ATG

GLN GLY SER ALA ALA VAL ALA ALA GLU \*\*\*  
 CAA GGC TCG GCT GCG GTC GCG GCC GAG TAA TCGTGCSCAAAGCCGCGGCTG  
 GTT CCG AGC CGA CCG CAG GCG CCG CTC ATT ACGACGCGTTCGCGCCGAGC

CGGGCCACGCGCAAGCCAGTCCGTSAGTCCGAGCAGGCGCGGA  
 GCGCGGGTGGGTGCGGTCAAGCACTCAGGCTCGTCCGCGCTT

Fig.3. Nucleotide and deduced amino acid sequence of the genes encoding B800-850  $\alpha$  and  $\beta$  polypeptides.

### 3.2. Transfer of *pucA* and *B* genes to mutant M21

In order to test whether these genes could restore B800-850 to a mutant lacking this light-harvesting complex, mutant M21 was isolated. This mutant has been characterised by spectroscopy at 4 K [15] and the amount of B800-850 complex present has been determined by circular dichroism to be less than 1% (Van Dorssen, R., Van Grondelle, R. and C.N.H., unpublished). This figure is in agreement with results from hybridisation analyses of mRNA from M21 probed with the radiolabelled 1.1 kb *Bam*HI fragment purified from pMA81, where the level of transcription of *pucA* and *B* genes was at the limit of detection. Fig.4 shows three absorbance spectra, including a control in which the cloning vector alone (no insert) was transferred to the wild-type. This spectrum exhibits characteristic peaks at 800 and 850 nm (attributable to B800-850 antenna) and a shoulder at 875 nm (B875 antenna). M21, which lacks B800-850, shows only a peak at 875 nm and a small maximum at 800 nm which is due to some of the reaction centre

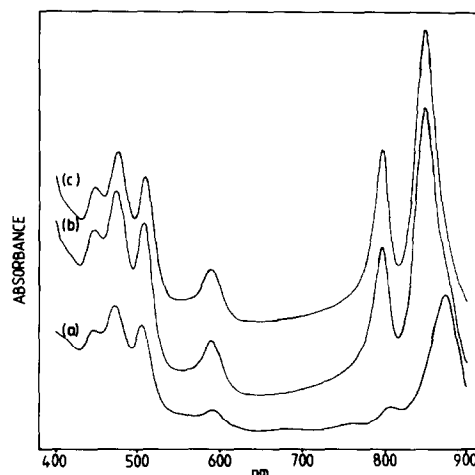


Fig.4. Absorbance spectra of (a) mutant M21 and (b) transconjugant strain M2181, with (c) wild type containing cloning vector pNH2 included as a control.

bacteriochlorophylls and not residual B800; specific mutation of reaction centre genes in M21 results in the disappearance of this absorbance peak (Hunter, C.N., unpublished).

The strain M2181 obtained from transfer of pMA81 into M21 has an absorbance spectrum closely resembling that of the wild type showing that the transfer and expression of *pucA* and *B* genes have been achieved. Preliminary measurements of fluorescence emission at 4 K indicate that the efficiency of energy transfer from B850 to B875 is comparable to that in the wild-type (Van Dorssen, R., Van Grondelle, R. and C.N.H., unpublished). We have noted that the transfer of pMA84 into M21 (not shown) also results in expression of *pucA* and *B* genes although at reduced levels, an observation that we ascribe to some difficulty in maintenance of pMA84 which has a relatively large insert.

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